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12 near10 atp	4

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Refine Search:

12 near10 atp

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Today's Date: 12/11/2001

<u>DB Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
USPT	12 near10 atp	4	<u>L4</u>
USPT	luciferase adj5 muta\$4	73	<u>L3</u>
USPT	luciferase near5 muta\$4	110	<u>L2</u>
USPT	6074859	1	<u>L1</u>

**WEST****End of Result Set**☐ **Generate Collection**

L1: Entry 1 of 1

File: USPT

Jun 13, 2000

US-PAT-NO: 6074859

DOCUMENT-IDENTIFIER: US 6074859 A

TITLE: Mutant-type bioluminescent protein, and process for producing the mutant-type bioluminescent protein

DATE-ISSUED: June 13, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hirokawa; Kozo	Chiba			JPX
Kajiyama; Naoki	Chiba			JPX
Murakami; Seiji	Chiba			JPX

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Kikkoman Corporation	Noda			JPX	03

APPL-NO: 9/ 111752

DATE FILED: July 8, 1998

## PARENT-CASE:

This application claims benefit of priority under 35 U.S.C. .sctn. 119(e) to U.S. Provisional Application Serial No. 60/051,917, filed on Jul. 8, 1997.

INT-CL: [7] C12N 9/02

US-CL-ISSUED: 435/189; 435/440, 435/441, 435/69.1, 435/71.1, 435/71.2, 435/8, 530/858

US-CL-CURRENT: 435/189; 435/440, 435/441, 435/69.1, 435/71.1, 435/71.2, 435/8, 530/858

FIELD-OF-SEARCH: 435/8, 435/69.1, 435/71.1, 435/71.2, 435/440, 435/441, 435/189, 530/858

## PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

		Search Selected	Search ALL	
PAT-NO	ISSUE-DATE	PATENTEE-NAME		US-CL
<input type="checkbox"/> 5229285	July 1993	Kajiyama et al.		435/189
<input type="checkbox"/> 5843746	December 1998	Tatsumi et al.		435/189

## OTHER PUBLICATIONS

Kajiyama et al., Isolation and Characterization of Mutants of Firefly Luciferase Which Produce Different Colors of Light, Protein Engineering, 4 (6):691-693, 1991.  
Wood et al., Bioluminescent Click Beetles Revisited, J. Biolumin. Chemilum. 4:

31-39, Jul. 1989.

De Wet et al., Cloning of Firefly Luciferase cDNA and the Expression of Active Luciferase in *Escherichia coli*, PNAS 82: 7870-7873, Dec. 1985.

Masuda et al. Cloning and Sequence Analysis of cDNA for Luciferase of a Japanese Firefly, *Luciola cruciatea*, Gene 77: 265-270, 1989.

Kajuyama et al., Purification and Characterization of Luciferases from Fireflies, *Luciola cruciata* and *Luciola lateralis*, Biochim. Biophys. Acta. 1120:228-232, 1992.

ART-UNIT: 162

PRIMARY-EXAMINER: Prouty; Rebecca E.

ASSISTANT-EXAMINER: Hutson; Richard

ATTY-AGENT-FIRM: Oblon, Spivak, McClelland, Maier & Neustadt, P.C.

ABSTRACT:

According to the present invention, there can be provided a bioluminescent protein, luciferase excellent in thermostability etc. and with high catalytic efficiency.

12 Claims, 1 Drawing figures

**WEST****End of Result Set**☐ **Generate Collection**

L1: Entry 1 of 1

File: USPT

Jun 13, 2000

US-PAT-NO: 6074859

DOCUMENT-IDENTIFIER: US 6074859 A

TITLE: Mutant-type bioluminescent protein, and process for producing the mutant-type bioluminescent protein

DATE-ISSUED: June 13, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hirokawa; Kozo	Chiba			JPX
Kajiyama; Naoki	Chiba			JPX
Murakami; Seiji	Chiba			JPX

US-CL-CURRENT: 435/189; 435/440, 435/441, 435/69.1, 435/71.1, 435/71.2, 435/8,  
530/858

## CLAIMS:

What is claimed is:

1. A bioluminescent protein having firefly luciferase activity and having a mutation in an amino acid residue corresponding to the 219-position of the *Luciola cruciata* luciferase.
2. The bioluminescent protein of claim 1, wherein the amino C acid residue corresponding to the 219-position of the *Luciola cruciata* luciferase is an isoleucine residue.
3. A bioluminescent protein having firefly luciferase activity and having a mutation in an amino acid residue corresponding to the 290-position of the *Luciola cruciata* luciferase.
4. The bioluminescent protein of claim 3, wherein the amino C acid residue corresponding to the 290-position of the *Luciola cruciata* luciferase is an isoleucine residue.
5. A bioluminescent protein having firefly luciferase activity, wherein the bioluminescent protein of claim 1 is fused to at least one other bioluminescent protein having firefly luciferase activity.
6. The bioluminescent protein of claim 5, wherein said other bioluminescent protein having firefly luciferase activity is a luciferase from Heike firefly (*Luciola lateralis*), American firefly (*Photinus pyralis*) or Genji firefly (*Luciola cruciata*).
7. A bioluminescent protein having firefly luciferase activity, wherein the bioluminescent protein of claim 2 is fused to at least one other bioluminescent protein having firefly luciferase activity.
8. The bioluminescent protein of claim 7, wherein said other bioluminescent protein having firefly luciferase activity is a luciferase from Heike firefly (*Luciola lateralis*), American firefly (*Photinus pyralis*) or Genji firefly (*Luciola cruciata*).
9. A bioluminescent protein having firefly luciferase activity, wherein the bioluminescent protein of claim 3 is fused to at least one other bioluminescent protein having firefly luciferase activity.
10. The bioluminescent protein of claim 9, wherein said other bioluminescent protein having firefly luciferase activity is a luciferase from Heike firefly (*Luciola lateralis*), American firefly (*Photinus pyralis*) or Genji firefly (*Luciola cruciata*).

(*Luciola cruciata*).

11. A bioluminescent protein having firefly luciferase activity, wherein the bioluminescent protein of claim 4 fused to at least one other bioluminescent protein having firefly luciferase activity.
12. The bioluminescent protein of claim 11, wherein said other bioluminescent protein having firefly luciferase activity is a luciferase from Heike firefly (*Luciola lateralis*), American firefly (*Photinus pyralis*) or Genji firefly (*Luciola cruciata*).

**WEST**☐ Generate Collection

L4: Entry 1 of 4

File: USPT

Jul 24, 2001

US-PAT-NO: 6265177

DOCUMENT-IDENTIFIER: US 6265177 B1

TITLE: Enzyme assay for mutant firefly luciferase

DATE-ISSUED: July 24, 2001

## INVENTOR-INFORMATION:

NAME

Squirrell; David James

White; Peter John

Lowe; Christopher Robin

Murray; James Augustus Henry

CITY

Salisbury

Cambridge

Cambridge

Cambridge

STATE ZIP CODE

COUNTRY

GBX

GBX

GBX

GBX

## ASSIGNEE-INFORMATION:

NAME

The United States of America as  
represented by the Secretary of the State  
of Defence of Defence

CITY STATE ZIP CODE COUNTRY TYPE CODE

APPL-NO: 9/ 380061

DATE FILED: August 25, 1999

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY

APPL-NO

APPL-DATE

GB

9707486

April 11, 1997

## PCT-DATA:

APPL-NO

DATE-FILED

PUB-NO

PUB-DATE

371-DATE

102 (E) -DATE

PCT/GB98/01026 April 7, 1998 WO98/46729 Oct 22, 1998 Aug 25, 1999 Aug 25, 1999

INT-CL: [7] C12Q 1/66, C12N 9/02, C12N 1/21, C12N 15/52, C07H 21/04  
US-CL-ISSUED: 435/8; 435/189, 435/252.3, 435/320.1, 435/440, 435/810, 536/23.2  
US-CL-CURRENT: 435/8; 435/189, 435/252.3, 435/320.1, 435/440, 435/810, 536/23.2  
FIELD-OF-SEARCH: 435/189, 435/320.1, 435/252.3, 435/810, 435/440, 536/23.2

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected

Search ALL

PAT-NO

ISSUE-DATE

PATENTEE-NAME

US-CL

☐ 5196524

March 1993

Gustafson et al.

536/23.2

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 449 621	October 1991	EPX	
WO 95 18853	July 1995	WOX	
WO 95 25798	September 1995	WOX	
WO 96 22376	July 1996	WOX	

## OTHER PUBLICATIONS

Dementieva et al, "Physicochemical properties of recombinant *Luciola mingrelica* luciferase and its mutant forms" Biochemistry, vol. 61, No. 1, 1996, pp. 115-119.  
Dementieva et al, "Assay of ATP in intact *Escherichia coli* cells expressing recombinant firefly luciferase" Biochemistry, vol. 61, No. 7, 1996, pp. 915-920.  
Liu et al, "Factors influencing the efficiency of cationic liposome-mediated intravenous gene delivery", Nature Biotechnology, vol. 15, 1997, pp. 167-173.

ART-UNIT: 162

PRIMARY-EXAMINER: Slobodyansky; Elizabeth

ATTY-AGENT-FIRM: Nixon &amp; Vanderhye P.C.

## ABSTRACT:

Enzymes and methods suitable for assaying ATP, and specific application for such assays are described and claimed. In particular, there is described a recombinant mutant luciferase having a mutation for example, in the amino-acid corresponding to amino acid residue number 245 in *Photinus pyralis*, is such that the  $K_{sub.m}$  for ATP of the luciferase is increased e.g. five-fold with respect to that of the corresponding non-mutated enzyme such that it is of the order of 500  $\mu\text{M}$ . Also disclosed are luciferases having additional mutations conferring improved thermostability or altered wavelength of emitted light. Recombinant polynucleotides, vectors and host cells are also disclosed, as are methods of assaying the amount of ATP in a material (e.g. cells) optionally in real-time. Also disclosed are test-kits for in vitro assays.

34 Claims, 11 Drawing figures

**WEST**☐ Generate Collection

L4: Entry 1 of 4

File: USPT

Jul 24, 2001

US-PAT-NO: 6265177

DOCUMENT-IDENTIFIER: US 6265177 B1

TITLE: Enzyme assay for mutant firefly luciferase

DATE-ISSUED: July 24, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Squirrell; David James	Salisbury			GBX
White; Peter John	Cambridge			GBX
Lowe; Christopher Robin	Cambridge			GBX
Murray; James Augustus Henry	Cambridge			GBX

US-CL-CURRENT: 435/8; 435/189, 435/252.3, 435/320.1, 435/440, 435/810, 536/23.2

## CLAIMS:

What is claimed is:

1. A recombinant mutant luciferase having 70% or more homology to a luciferase of Photinus pyralis (SEQ ID NO: 21), Luciola cruciata (SEQ ID NO:14), Luciola lateralis (SEQ ID NO:16), Luciola mingrelia (SEQ ID NO:18) or Lampyris noctiluca (SEQ ID NO:20); wherein the amino-acid corresponding to amino acid residue number 245 or 318 in Photinus pyralis luciferase has been substituted with respect to the corresponding wild-type amino acid residue such that the K.sub.m for ATP is increased with respect to that of the corresponding non-mutated enzyme.
2. A recombinant mutant luciferase according to claim 1 wherein the amino-acid corresponding to amino acid residue number 245 in Photinus pyralis luciferase has been substituted with respect to the corresponding wild-type amino acid residue.
3. A luciferase as claimed in claim 1 wherein the K.sub.m is at least double that of the non-mutated enzyme.
4. A luciferase as claimed in claim 3 wherein the K.sub.m is at least five times higher than that of the non-mutated enzyme.
5. A luciferase as claimed in claim 1 wherein the K.sub.m is of the order of 500 .mu.m.
6. A luciferase as claimed in claim 1 wherein the K.sub.m is of the order of 1 mM.
7. A luciferase as claimed in claim 1 having a V.sub.m for ATP which is 5-100% of that of the corresponding wild-type.
8. A luciferase as claimed in claim 2 wherein the said amino-acid has been substituted for an uncharged amino acid.
9. A luciferase as claimed in claim 7 wherein the amino-acid has been substituted for Ala, Asn, or Gln.
10. A luciferase as claimed in claim 1 which is derived from Photinus pyralis and wherein amino acid residue number 245 has, been substituted.
11. A luciferase as claimed in claim 1 which is derived from Luciola cruciata and wherein amino acid residue number 247 has been substituted.
12. A luciferase as claimed in claim 1 that includes one or more mutations capable of conferring one or more of the following properties with respect to a corresponding non-mutated enzyme: improved thermostability; or, altered wavelength of emitted light.
13. A fusion protein comprising a luciferase as claimed in claim 1.
14. A recombinant polynucleotide encoding a luciferase as claimed in claim 1.



15. A replication vector comprising a polynucleotide as claimed in claim 14 further comprising a replication element which permits replication of the vector in a suitable host cell.
16. An expression vector comprising a polynucleotide as claimed in claim 14 further comprising a promoter element which permits expression of said polynucleotide in a suitable host cell.
17. A vector as claimed in claim 16 wherein the promoter element is tissue or organ specific.
18. A host cell containing a vector as claimed in claim 15.
19. A host cell transformed with a vector as claimed in claim 15.
20. A host cell as claimed in claim 19 which also expresses a second luciferase having a lower  $K_{sub.m}$  for ATP.
21. A host cell as claimed in claim 20 wherein the second luciferase is selected from: (a) a recombinant non-mutant luciferase; and (b) a recombinant mutant luciferase having a mutation which is such that the  $K_{sub.m}$  for ATP of the luciferase is decreased with respect to that of the corresponding non-mutated enzyme.
22. A process for producing a luciferase comprising culturing a host cell as claimed in claim 19.
23. A method of assaying the amount of ATP in a material, said method comprising the steps of (a) contacting a recombinant mutant luciferase of claim 1 with the material and luciferin; (b) measuring the intensity of light emitted by the luciferase; and (c) the measurement in step (b) is correlated directly with the amount of ATP in the material.
24. A method according to claim 23 wherein the concentration of the ATP in the material is expected to be between 300  $\mu M$  and 6 mM.
25. A method according to claim 23 wherein step (c) is effected by comparison of the measurement obtained in step (b) with a control value.
26. A method as claimed in claim 23 wherein the measurement in step (b) is monitored continuously.
27. A method as claimed in claim 23 wherein the material measured is a cell which forms part of a synapse.
28. A method as claimed in claim 23 wherein the material is a cell and the luciferase is introduced into the cell.
29. A method as claimed in claim 28 wherein the luciferin is introduced into the cell by direct injection.
30. A method as claimed in claim 28 wherein the luciferase is introduced into the cell by transforming the cell with a vector comprising a polynucleotide which encodes a recombinant mutant luciferase.
31. A method of producing a mutant luciferase with an increased Michaelis-Menten constant ( $K_{sub.m}$ ) for the substrate ATP of a luciferase enzyme having 70% or more homology to luciferase of Photinus pyralis (SEQ ID NO: 21), Luciola cruciata (SEQ ID NO:14), Luciola lateralis (SEQ ID NO:16), Luciola mingrelica (SEQ ID NO:18) or Lampyrus noctiluca (SEQ ID NO:20); said method comprising mutating an amino acid residue of said luciferase corresponding to residue 245 or 318 of Photinus pyralis luciferase.
32. A luciferase produced by the method of claim 31.
33. In a luciferase having 70% or more homology to luciferase of Photinus pyralis (SEQ ID NO: 21), Luciola cruciata (SEQ ID NO:14), Luciola lateralis (SEQ ID NO:16), Luciola mingrelica (SEQ ID NO:18) or Lampyrus noctiluca (SEQ ID NO:20); the improvement comprising a mutated amino acid at the amino acid residue corresponding to residue 245 or 318 of Photinus pyralis luciferase, wherein said improved luciferase has a  $K_{sub.m}$  for the substrate ATP which is higher than that of the wild type luciferase.
34. A test kit comprising a luciferase as claimed in claim 1 and further comprising one or more of the following (a) a buffer or dry materials for preparing a buffer; (b) two or more measured portions of ATP suitable for preparing standard solutions; (c) luciferin; (d) instructions for carrying out an ATP assay.

**WEST**☐ Generate Collection

L4: Entry 2 of 4

File: USPT

Jan 9, 2001

US-PAT-NO: 6171808  
DOCUMENT-IDENTIFIER: US 6171808 B1

TITLE: Mutant luciferases

DATE-ISSUED: January 9, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Squirrell; David J	Salisbury			GBX
Lowe; Christopher R	Cambridge			GBX
White; Peter J	Cambridge			GBX
Murray; James A H	Cambridge			GBX

## ASSIGNEE-INFORMATION:

NAME

CITY STATE ZIP CODE COUNTRY TYPE CODE

The Secretary of State for Defence in Her  
Britannic Majesty's Government of the  
United Kingdom of Great Britain and  
Northern Ireland the United Kingdom of  
Great Britain and Northern Ireland

APPL-NO: 8/ 875277

DATE FILED: October 1, 1997

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9501172	January 20, 1995
GB	9508301	April 24, 1995

## PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/GB96/00099	January 19, 1996	WO96/22376	Jul 25, 1996	Oct 1, 1997	Oct 1, 1997

INT-CL: [7] C12N 9/02, C12N 15/53, C12N 1/21, C12Q 1/66  
US-CL-ISSUED: 435/8; 435/189, 435/320.1, 435/252.3, 435/252.33, 435/254.21,  
536/23.2  
US-CL-CURRENT: 435/8; 435/189, 435/252.3, 435/252.33, 435/254.21, 435/320.1,  
536/23.2  
FIELD-OF-SEARCH: 435/189, 435/320.1, 435/252.33, 435/254.21, 435/8, 435/252.3,  
536/23.2

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

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PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
5229285	July 1993	Kajiyama et al.	435/189

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 449 621	October 1991	EPX	
0 524 488	January 1993	EPX	
95 18853	July 1995	WOX	

## OTHER PUBLICATIONS

Wood, K.V. et al. "Complementary DNA coding click beetle luciferases can elicit bioluminescence of different colors." Science (May 1989), vol. 244, pp. 700-702.

Kajiyama, N. et al. "Thermostabilization of firefly luciferase by a single amino acid substitution at position 217." Biochemistry (Dec. 1993), vol. 32, No. 50, pp. 13795-13799.

ART-UNIT: 162  
PRIMARY-EXAMINER: Prouty; Rebecca E.  
ATTY-AGENT-FIRM: Nixon & Vanderhye PC

## ABSTRACT:

Proteins are provided having luciferase activity with lower  $K_{sub.m}$  than wild-type luciferases by altering the amino acid residue at position 270 of the wild-type to an amino acid other than glutamate. Greater heat stability than wild-type luciferases while retaining the lower  $K_{sub.m}$  is provided by also replacing the glutamate equivalent to that at position 354 of Photinus pyralis luciferase or 356 of Luciola luciferases with an alternative amino acid, particularly lysine and/or the amino acid residue at 215 of Photinus pyralis and 217 of the Luciola species with a hydrophobic amino acid. DNA, vectors and cells that encode for and express the proteins are also provided as are test kits and reagents for carrying out luminescence assays using the proteins of the invention.

25 Claims, 6 Drawing figures

**WEST**☐ Generate Collection

L4: Entry 2 of 4

File: USPT

Jan 9, 2001

US-PAT-NO: 6171808

DOCUMENT-IDENTIFIER: US 6171808 B1

TITLE: Mutant luciferases

DATE-ISSUED: January 9, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Squirrell; David J	Salisbury			GBX
Lowe; Christopher R	Cambridge			GBX
White; Peter J	Cambridge			GBX
Murray; James A H	Cambridge			GBX

US-CL-CURRENT: 435/8; 435/189, 435/252.3, 435/252.33, 435/254.21, 435/320.1,  
536/23.2

## CLAIMS:

What is claimed is:

1. An isolated mutant luciferase protein having luciferase activity, which has over 60% amino acid sequence homology to the luciferase from Photinus pyralis, Luciola mingrelica, Luciola cruciata or Luciola lateralis and which includes the amino acid sequence F(1)XE(2)FL (SEQ ID NO: 6), where (1) is D or E, (2) is T or L and X is an amino acid other than glutamate and is at a position corresponding to amino acid residue 270 of Photinus pyralis luciferase as shown in SEQ ID NO: 2.
2. A protein as claimed in claim 1 wherein it further comprises an amino acid sequence TPXGDDKPGA (SEQ ID NO. 7) wherein X is an amino acid residue other than glutamate.
3. A protein as claimed in claim 1, wherein the amino acid residue X is lysine.
4. A protein as claimed in claim 1 wherein the amino acid residue corresponding to residue 215 of Photinus pyralis luciferase is a hydrophobic amino acid.
5. A protein as claimed in claim 4 wherein the residue corresponding to residue 215 of Photinus pyralis luciferase is one of isoleucine, leucine or valine.
6. A protein as claimed in claim 1 wherein the amino acid residue corresponding to residue 354 of Photinus pyralis luciferase is an amino acid other than glutamate.
7. A protein of claim 6 wherein the residue corresponding to residue 354 of Photinus pyralis luciferase is one of lysine, arginine, leucine, isoleucine or histidine.
8. An isolated DNA encoding for a protein as claimed in claim 1.
9. An isolated DNA as claimed in claim 8 comprising a nucleotide sequence as described in SEQ ID No 1 wherein nucleotide residues 811-813 form a codon encoding an amino acid other than glutamate.
10. An isolated DNA as claimed in claim 9 wherein the codon encodes lysine.
11. A vector comprising a luc gene encoding a protein as claimed in claim 1.
12. A vector as claimed in claim 11 obtainable by treating a vector containing a wild-type or recombinant luc gene by site directed mutagenesis to change the codon responsible for encoding the glutamate at position 270 of Photinus pyralis luciferase to an alternative amino acid.
13. A vector as claimed in claim 12 wherein the alternative amino acid is lysine.

14. A vector as claimed in claim 11 selected from pKK223-3, pDR540 and pT7-7 into which said luc gene has been ligated.
15. A cell transformed with a DNA or a vector capable of expressing a protein as claimed in claim 1.
16. A cell as claimed in claim 15 which is an E. coli or a S. cerevisiae cell.
17. A test kit for performance of an assay through measurement of ATP wherein the kit comprises a protein as claimed in claim 1.
18. An assay method wherein ATP is measured using luciferin and luciferase to generate light the quantity of which is related to the amount of ATP wherein the luciferase is a protein as claimed in claim 1.
19. An assay method as claimed in claim 18 wherein the assay is carried out at a temperature of from 30.degree. C. to 70.degree. C.
20. An assay method as claimed in claim 18 wherein the assay is carried out at a temperature of from 37.degree. C. to 60.degree. C.
21. An assay method as claimed in claim 18 wherein the assay is carried out at a temperature of from 40.degree. C. to 50.degree. C.
22. A protein comprising an amino acid sequence as described in SEQ ID No 2 wherein Xaa is chosen from arginine, glutamine and alanine.
23. A mutant luciferase protein of claim 1 wherein said luciferase protein is a firefly or a glow worm luciferase.
24. A mutated luciferase of claim 1 wherein said luciferase is a Photinus luciferase.
25. A mutant luciferase protein as claimed in claim 1 wherein said luciferase has a K.sub.m to the substrate ATP which is lower than that of the corresponding wild type luciferase.

**WEST**☐ Generate Collection

L4: Entry 3 of 4

File: USPT

Jul 19, 1994

US-PAT-NO: 5330906  
DOCUMENT-IDENTIFIER: US 5330906 A

TITLE: Mutant luciferase of a firefly

DATE-ISSUED: July 19, 1994

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kajiyama; Naoki	Noda			JPX
Nakano; Eiichi	Noda			JPX

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Kikkoman Corporation				JPX	03

APPL-NO: 8/ 076042  
DATE FILED: June 15, 1993

PARENT-CASE:  
This is a division of application Ser. No. 07/675,211, filed Mar. 26, 1991, now  
U.S. Pat. No. 5,219,737.

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	2-75696	March 27, 1990
JP	2-294258	October 30, 1990

INT-CL: [5] C12N 9/02, C12N 15/53, C12N 15/70  
US-CL-ISSUED: 435/189, 435/71.2, 435/172.3, 435/252.3, 435/252.33, 435/320.1,  
935/10, 935/14, 935/27, 935/56, 536/23.2  
US-CL-CURRENT: 435/189, 435/252.3, 435/252.33, 435/320.1, 435/71.2, 536/23.2  
FIELD-OF-SEARCH: 435/189, 435/71.2, 435/172.3, 435/252.3, 435/252.33, 435/320.1,  
935/10, 935/14, 935/27, 935/56, 536/23.2

## PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

☐ Search Selected☐ Search ALL

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> 4320195	March 1982	Hill et al.	435/55
<input type="checkbox"/> 4743561	March 1985	Shaffer	436/501

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO  
0301541  
0353464

PUBN-DATE  
July 1988  
February 1990

COUNTRY  
EPX  
EPX  
US-CL

## OTHER PUBLICATIONS

Masuda et al. Gene 77:265-270 (1989).  
Chu et al. Virology 98:161-181 (1979).  
DeWet et al. Mol. Cell. Biol 7:725-737 (Feb. 1987).  
DeWet et al., 1985, Proc. Natl. Acad. Sci, USA, 82:7870-7873.  
Wood et al., 1989, Science, 244:700-702.  
Wood et al., 1989, J. Bioluminescence and Chemiluminescence 4:289-301.  
Wood et al., 1989, J. Bioluminescence and Chemiluminescence 4:31-39.  
Wood, J. Bioluminescence and Chemiluminescence 5:107-114 (Paper associated with talk presented at: Symposium on Lux Genes: Basic, Applications and Commercial Prospects, Cambridge, U.K., Dec., 1989).

ART-UNIT: 184  
PRIMARY-EXAMINER: Patterson, Jr.; Charles L.  
ASSISTANT-EXAMINER: Prouty; Rebecca  
ATTY-AGENT-FIRM: Pennie & Edmonds

## ABSTRACT:

The present invention provides industrially useful luciferase. Mutant luciferase of the invention is produced by culturing a microorganism belonging to the genus *Escherichia* which harbors a recombinant DNA containing the mutant luciferase gene of a firefly. Mutant luciferase can produce red, orange or green color of light which can not be produced by wild type luciferase. Mutant luciferase can be used to measure ATP accurately in a colored solution such as red (e.g., blood), orange, or green in which wild-type luciferase has not provided reliable results.

5 Claims, 2 Drawing figures

**WEST**☐ Generate Collection

L4: Entry 3 of 4

File: USPT

Jul 19, 1994

US-PAT-NO: 5330906

DOCUMENT-IDENTIFIER: US 5330906 A

TITLE: Mutant luciferase of a firefly

DATE-ISSUED: July 19, 1994

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kajiyama; Naoki	Noda			JPX
Nakano; Eiichi	Noda			JPX

US-CL-CURRENT: 435/189; 435/252.3, 435/252.33, 435/320.1, 435/71.2, 536/23.2

## CLAIMS:

What is claimed is:

1. A mutant luciferase having the amino acid sequence of the luciferase of *Luciola cruciata* in which one of the following changes appears: serine is replaced by asparagine at amino acid 286, glycine is replaced by serine at amino acid 326, histidine is replaced by tyrosine at amino acid 433 or proline is replaced by serine at amino acid 452.
2. The mutant luciferase according to claim 1, in which serine is replaced by asparagine at amino acid 286.
3. The mutant luciferase according to claim 1, in which glycine is replaced by serine at amino acid 326.
4. The mutant luciferase according to claim 1, in which histidine is replaced by tyrosine at amino acid 433.
5. The mutant luciferase according to claim 1, in which proline is replaced by serine at amino acid 452.



**WEST****End of Result Set**☐ **Generate Collection**

L4: Entry 4 of 4

File: USPT

Jun 15, 1993

US-PAT-NO: 5219737  
DOCUMENT-IDENTIFIER: US 5219737 A

TITLE: Mutant luciferase of a firefly, mutant luciferase genes, recombinant DNAs  
containing the genes and a method of producing mutant luciferase

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Kajiyama; Naoki	Noda			JPX
Nakano; Eiichi	Noda			JPX

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Kikkoman Corporation	Chiba			JPX	03

APPL-NO: 7/ 675211  
DATE FILED: March 26, 1991

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	2-75696	March 27, 1990
JP	2-294258	October 30, 1990

INT-CL: [5] C12N 9/02, C12N 15/53  
US-CL-ISSUED: 435/69.1, 435/71.1, 435/71.2, 435/172.1, 435/172.3, 435/189,  
435/252.3, 435/252.33, 435/320.1, 536/23.2, 935/10, 935/14, 935/27, 935/56  
US-CL-CURRENT: 435/189, 435/252.3, 435/252.33, 435/320.1, 435/71.1, 435/71.2,  
536/23.2  
FIELD-OF-SEARCH: 435/8, 435/69.1, 435/71.1, 435/71.2, 435/172.1, 435/172.3,  
435/189, 935/10, 935/14, 935/27, 935/56, 536/27

PRIOR-ART-DISCLOSED:

## U.S. PATENT DOCUMENTS

	Search Selected	Search ALL
PAT-NO	ISSUE-DATE	PATENTEE-NAME
<input type="checkbox"/> <u>4320195</u>	March 1982	Hill et al.
<input type="checkbox"/> <u>4743561</u>	May 1988	Shaffer
		US-CL
		N/A
		436/501

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO  
0301541  
0353464

PUBN-DATE  
July 1988  
February 1990

COUNTRY  
EPX  
EPX  
US-CL

## OTHER PUBLICATIONS

Masuda et al. "Cloning and Sequence Analysis of CDNA . . . " Gene 77: 265-270 (1989).  
Chu et al. "Hydroxylamine Mutagenesis of HSV DNA . . . " Virology 98: 161-181 (1979).  
de Wet et al. "Firefly Luciferase Gene: Structure and . . . " Mol. and Cell. Biol. 7: 725-737 (Feb. 1987).  
Wood et al., 1989, J. Bioluminescence and Chemiluminescence 4:289-301.  
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Wood, J. Bioluminescence and Chemiluminescence 5:107-114 (Paper associated with talk presented at: Symposium on Lux Genes: Basics, Applications and Commercial Prospects, Cambridge, U.K., Dec. 1989).  
DeWet et al., 1985, Proc. Natl. Acad. Sci., USA, 82:7870-7873.  
Wood et al., 1989, Science, 244:700-702.

ART-UNIT: 184  
PRIMARY-EXAMINER: Wax; Robert A.  
ASSISTANT-EXAMINER: Prouty; Rebecca  
ATTY-AGENT-FIRM: Pennie & Edmonds

## ABSTRACT:

The present invention provides industrially useful luciferase. Mutant luciferase of the invention is produced by culturing a microorganism belonging to the genus *Escherichia* which harbors a recombinant DNA containing the mutant luciferase gene of a firefly. Mutant luciferase can produce red, orange or green color of light which can not be produced by wild type luciferase. Mutant luciferase can be used to measure ATP accurately in a colored solution such as red (e.g., blood), orange, or green in which wild-type luciferase has not provided reliable results.

15 Claims, 2 Drawing figures

**WEST****End of Result Set**☐ Generate Collection

L4: Entry 4 of 4

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## CLAIMS:

What is claimed is:

1. A mutant luciferase gene encoding the amino acid sequence of luciferase of *Luciola cruciata*, in which one of the following changes appears: serine is replaced by asparagine at amino acid 286, glycine is replaced by serine at amino acid 326, histidine is replaced by tyrosine at amino acid 433 or proline is replaced by serine at amino acid 452.
2. The mutant luciferase gene according to claim 1, in which serine is replaced by asparagine at amino acid 286.
3. The mutant luciferase gene according to claim 1, in which glycine is replaced by serine at amino acid 326.
4. The mutant luciferase gene according to claim 1, in which histidine is replaced by tyrosine at amino acid 433.
5. The mutant luciferase gene according to claim 1, in which proline is replaced by serine at amino acid 452.
6. A recombinant DNA comprising the mutant luciferase gene of claim 1.
7. A recombinant DNA comprising the mutant luciferase gene of claim 2.
8. A recombinant DNA comprising the mutant luciferase gene of claim 3.
9. A recombinant DNA comprising the mutant luciferase gene of claim 4.
10. A recombinant DNA comprising the mutant luciferase gene of claim 5.
11. A method of producing a mutant firefly luciferase, which comprises culturing, in a culture medium, a microorganism belonging to the genus *Escherichia* transformed with a recombinant DNA containing a mutant gene encoding the amino acid sequence of luciferase of *Luciola cruciata*, in which one of the following changes appears: serine is replaced by asparagine at amino acid 286, glycine is replaced by serine at amino acid 326, histidine is replaced by tyrosine at amino acid 433 or proline is replaced by serine at amino acid 452, and recovering the mutant luciferase from the culture.
12. The method according to claim 11, in which the microorganism contains recombinant DNA having the mutant gene encoding the amino acid sequence of *Luciola cruciata* luciferase in which serine is replaced by asparagine at amino acid 286.
13. The method according to claim 11, in which the microorganism contains recombinant DNA having the mutant gene encoding the amino acid sequence of *Luciola cruciata* luciferase in which glycine is replaced by serine at amino acid 326.

14. The method according to claim 11, in which the microorganism contains recombinant DNA having the mutant gene encoding the amino acid sequence of *Luciola cruciata* luciferase in which histidine is replaced by tyrosine at amino acid 433.
15. The method according to claim 11, in which the microorganism contains recombinant DNA having the mutant gene encoding the amino acid sequence of *Luciola cruciata* luciferase in which proline is replaced by serine at amino acid 452.